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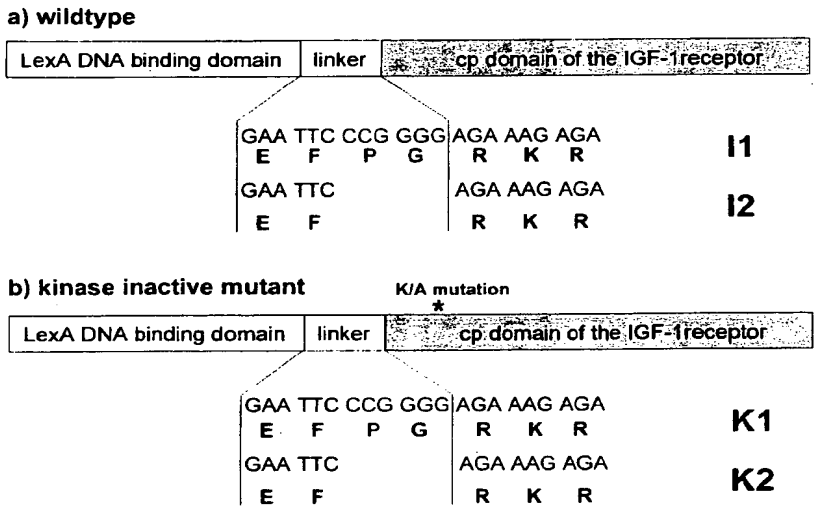
(54) IGF-1 receptor interacting proteins (IIPs) genes coding therefor and uses thereof

(57) The invention comprises an isolated nucleic acid molecule with the sequence SEQ ID NO:1 and the complementary sequence, and its use in diagnosis and therapy. This nucleic acid molecule is a gene which codes for an IGF-1 receptor binding polypeptide.

Fig. 1



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Description

[0001] The present invention relates to IGF-1 receptor interacting proteins (IIPs), nucleic acids coding therefor, their use for diagnostics and therapeutics, especially in the field of cancer. In particular, the invention relates to the diagnosis of said genes in mammalian cells, especially in malignant tumor cells, to gene therapy methods for inhibiting the interaction between IGF-1 receptor and IIPs, methods of screening for potential cancer therapy agents, and cell lines and animal models useful in screening for and evaluating potential useful pharmaceutical agents inhibiting the interaction between IIPs and IGF-1 receptor.

[0002] The present invention relates in particular to the cloning and characterization of the genes IIP-1 to IIP-110 and the gene products thereof. Said gene products (polypeptides, mRNA) are especially characterized as having the ability to modulate the IGF-1 receptor signaling pathway. The function of the gene products according to the invention is therefore to modulate signal transduction of the IGF-1 receptor. Forced activation of IIPs therefore correlates with increased tumor cell proliferation, survival and escape of apoptosis.

[0003] The IGF-1 receptor signaling system plays an important role in tumor proliferation and survival and is implicated in inhibition of tumor apoptosis. In addition and independent of its mitogenic properties, IGF-1R activation can protect against or at least retard programmed cell death in vitro and in vivo (Harrington et al., EMBO J. 13 (1994) 3286-3295; Sell et al., Cancer Res. 55 (1995) 303-305; Singleton et al., Cancer Res. 56 (1996) 4522-4529). A decrease in the level of IGF-1R below wild type levels was also shown to cause massive apoptosis of tumor cells in vivo (Resnicoff et al., Cancer Res. 55 (1995) 2463-2469; Resnicoff et al., Cancer Res. 55 (1995) 3739-3741). Overexpression of either ligand (IGF) and/or the receptor is a feature of various tumor cell lines and can lead to tumor formation in animal models. Overexpression of human IGF-1R resulted in ligand-dependent anchorage-independent growth of NIH 3T3 or Rat-1 fibroblasts and inoculation of these cells caused a rapid tumor formation in nude mice (Kaleko et al., Mol. Cell. Biol. 10 (1990) 464-473; Prager et al., Proc. Natl. Acad. Sci. USA 91 (1994) 2181-2185). Transgenic mice overexpressing IGF-II specifically in the mammary gland develop mammary adenocarcinoma (Bates et al., Br. J. Cancer 72 (1995) 1189-1193) and transgenic mice overexpressing IGF-II under the control of a more general promoter develop an elevated number and wide spectrum of tumor types (Rogler et al., J. Biol. Chem. 269 (1994) 13779-13784). One example among many for human tumors overexpressing IGF-I or IGF-II at very high frequency (>80%) are Small Cell Lung Carcinomas (Quinn et al., J. Biol. Chem. 271 (1996) 11477-11483). Signaling by the IGF system seems to be also required for the transforming activity of certain oncogenes. Fetal fibroblasts with a disruption of the IGF-1R gene cannot be transformed by the SV40 T antigen, activated Ha-ras, a combination of both (Sell et al., Proc. Natl. Acad. Sci. USA 90 (1993) 11217-11221; Sell et al., Mol. Cell. Biol. 14 (1994) 3604-3612), and also the E5 protein of the bovine papilloma virus is no longer transforming (Morrione et al., J. Virol. 69 (1995) 5300-5303). Interference with the IGF/IGF-1R system was also shown to reverse the transformed phenotype and to inhibit tumor growth (Trojan et al., Science 259 (1993) 94-97; Kalebic et al., Cancer Res. 54 (1994) 5531-5534; Prager et al., Proc. Natl. Acad. Sci. USA 91(1994) 2181-2185; Resnicoff et al., Cancer Res. 54 (1994) 2218-2222; Resnicoff et al., Cancer Res. 54 (1994) 4848-4850; Resnicoff et al., Cancer Res. 55 (1995) 2463-2469). For example, mice injected with rat prostate adenocarcinoma cells (PA-III) transfected with IGF-1R antisense cDNA (729 bp) develop tumors 90% smaller than controls or remained tumor-free after 60 days of observation (Burfeind et al., Proc. Natl. Acad. Sci. USA 93 (1996) 7263-7268). IGF-1R mediated protection against apoptosis is independent of de-novo gene expression and protein synthesis. Thus, IGF-1 likely exerts its anti-apoptotic function via the activation of preformed cytosolic mediators.

[0004] Some signaling substrates which bind to the IGF-1R (e.g. IRS-1, SHC, p85 PI3 kinase etc., for details see below) have been described. However, none of these transducers is unique to the IGF-1R and thus could be exclusively responsible for the unique biological features of the IGF-1R compared to other receptor tyrosine kinase including the insulin receptor. This indicates that specific targets of the IGF-1R (or at least the IGF-receptor subfamily) might exist which trigger survival and counteract apoptosis and thus are prime pharmaceutical targets for anti-cancer therapy.

[0005] By using the yeast two-hybrid system it was shown that p85, the regulatory domain of phosphatidyl inositol 3 kinase (PI3K), interacts with the IGF-1R (Lamothe, B., et al., FEBS Lett. 373 (1995) 51-55; Tartare-Decker, S., et al., Endocrinology 137 (1996) 1019-1024). However binding of p85 is also seen to many other receptor tyrosine kinases of virtually all families. Another binding partner of the IGF-1R defined by two-hybrid screening is SHC which binds also to other tyrosine kinases as trk, met, EGF-R and the insulin receptor (Tartare-Deckert, S., et al., J. Biol. Chem. 270 (1995) 23456-23460). The insulin receptor substrate 1 (IRS-1) and insulin receptor substrate 2 (IRS-2) were also found to interact both with the IGF-1R as well as the insulin receptor (Tartare-Deckert, S., et al., J. Biol. Chem. 270 (1995) 23456-23460; He, W., et al., J. Biol. Chem. 271 (1996) 11641-11645; Dey, R.B., et al., Mol. Endocrinol. 10 (1996) 631-641). Grb 10 which interacts with the IGF-1R also shares many tyrosine kinases as binding partners, e.g. met, insulin receptor, kit and abl (Dey, R.B., et al., Mol. Endocrinol. 10 (1996) 631-641; Morrione, A.) et al., Cancer Res. 56 (1996) 3165-3167). The phosphatase PTP1D (syp) shows also a very promiscuous binding capacity, i.e. binds to IGF-1R, insulin receptor, met and others (Rocchi, S., et al., Endocrinology 137 (1996) 4944-4952). More recently, mSH2-B and vav were described as binders of the IGF-1R, but interaction is also seen with other tyrosine kinases, e.g. mSH2-B also bind

to ret and the insulin receptor (Wang, J., and Riedel, H., J. Biol. Chem. 273 (1998) 3136-3139). Taken together, the so far described IGF-1R binding proteins represent relatively unspecific targets for therapeutic approaches, or are in the case of the insulin receptor substrates (IRS-1, IRS-2) indispensable for insulin-driven activities.

[0006] It is an object of the invention to provide novel genes encoding binding proteins of IGF-1R as well as the corresponding polypeptides which are the basis for new cancer therapy based on the modulation (preferably, inhibition) of the interaction between IGF-1R and IIPs according to the invention.

[0007] The invention preferably comprises an isolated nucleic acid molecule coding for a protein binding to IGF-1 receptor (IIP-1) selected from the group comprising

a) the nucleic acids shown in SEQ ID NO:1 or a nucleic acid sequence which is complementary thereto, or
b) nucleic acids which hybridize under stringent conditions with one of the nucleic acids from a).

[0008] „Interaction or binding between IIPs and the IGF-1 receptor" means a specific binding of the IIPs to the IGF-1 receptor but not to control proteins such as lamin in the yeast two hybrid system. Specific binding to the IGF-1 receptor can be demonstrated using glutathion-S-transferase (GST)-IIP fusion proteins expressed in bacteria and IGF-1 receptors expressed in mammalian cells. Furthermore, an association between a Flag tagged IIP-1 fusion protein (cf. Weidner, M., et al., Nature 384 (1996) 173-176) and the IGF-1 receptor can be monitored in mammalian cell systems. For this purpose eukaryotic expression vectors are used to transfect the respective cDNAs. Interaction between the proteins is visualized by coimmunoprecipitation experiments or subcellular localization studies using anti-Flag or anti-IGF-1 receptor antibodies.

[0009] Further provided by the invention are probes and primers for the genes according to the invention as well as nucleic acids which encode antigenic determinants of the gene products according to the invention. Therefore preferred embodiments include nucleic acids with preferably 10 to 50, or more preferably, 10 to 20 consecutive nucleotides out of the disclosed sequences.

[0010] The term "nucleic acid molecule" denotes a polynucleotide which can be, for example, a DNA, RNA, or derivatized active DNA or RNA. DNA and/or RNA molecules are preferred, however.

[0011] The term "isolated" as used throughout this application refers to a nucleic acid or polypeptide having IIP activity and is substantially free of cellular material or culture medium, when produced by recombinant DNA techniques, or chemical precursors or other chemicals, when synthesized chemically. An isolated nucleic acid is preferably free of sequences which naturally flank the nucleic acid (i.e. sequences located at the 5' and the 3' ends of the nucleic acid) in the organism from which the nucleic acid is derived.

[0012] The term "hybridize under stringent conditions" means that two nucleic acid fragments are capable of hybridization to one another under standard hybridization conditions described in Sambrook et al., Molecular Cloning: A laboratory manual (1989) Cold Spring Harbor Laboratory Press, New York, USA.

[0013] More specifically, "stringent conditions" as used herein refer to hybridization in 6.0 x SSC at about 45°C, followed by a wash with 2.0 x SSC at 50°C. For selection of the stringency the salt concentration in the wash step can be selected, for example from about 2.0 x SSC at 50°C, for low stringency, to about 0.2 x SSC at 50°C, for high stringency. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperatures, about 22°C, to high stringency conditions at about 65°C.

[0014] The invention further comprises recombinant expression vectors which are suitable for the expression of IIP, recombinant host cells transfected with such expression vectors, as well as a process for the recombinant production of a protein which is encoded by the IIP gene.

[0015] The invention further comprises synthetic and recombinant polypeptides which are encoded by the nucleic acids according to the invention, and preferably encoded by the DNA sequence shown in SEQ ID NO:1 as well as peptidomimetics based thereon. Such peptidomimetics have a high affinity for cell membranes and are readily taken up by the cells. Peptidomimetics are preferably compounds derived from peptides and proteins, and are obtained by structural modification using unnatural amino acids, conformational restraints, isosterical placement, cyclization, etc. They are based preferably on 24 or fewer, preferably 20 or fewer, amino acids, a basis of approximately 12 amino acids being particularly preferred.

[0016] The polypeptides and peptidomimetics can be defined by their corresponding DNA sequences and by the amino acid sequences derived therefrom. The isolated IIP polypeptide can occur in natural allelic variations which differ from individual to individual. Such variations of the amino acids are usually amino acid substitutions. However, they may also be deletions, insertions or additions of amino acids to the total sequence leading to biologically active fragments. The IIP protein according to the invention - depending, both in respect of the extent and type, on the cell and cell type in which it is expressed- can be in glycosylated or non-glycosylated form. Polypeptides with tumoricidic and/or metastatic activity can easily be identified by a tumor progression inhibition assay using carcinoma cells expressing said polypeptides and measuring the proliferation capacity and apoptosis in relation to carcinoma cells not expressing said polypeptides.

[0017] "Polypeptide with IIP activity or IIP" therefore means proteins with minor amino acid variations but with substantially the same IIP activity. Substantially the same means that the activities are of the same biological properties and the polypeptides show preferably at least 75% homology (identity) in amino acid sequences IIP-1, IIP-2, IIP-3, IIP-4, IIP-5, IIP-6, IIP-7, IIP-8, IIP-9, or IIP-10. More preferably, the amino acid sequences are at least 90% identical.

[0018] The IIPs according to the invention are in particular:

IIP-1

[0019] A novel cDNA encoding a new IGF-1 receptor interacting protein which was named IIP-1 (SEQ ID NO:1) was isolated. The cDNA of IIP-1 codes for a new protein of 333 aa with a calculated molecular weight of 35,727. IIP-1 is a glycine rich protein (13%). IIP-1 contains several N-myristoylation sites, PKC and Ck2 phosphorylation sites and two putative nuclear localization signals. A second isoform, IIP-1 (p26), of 236 aa in length with a calculated molecular weight of 26,071 was identified which was generated most likely by alternative splicing (Fig. 3). Both isoforms bind to the IGF-1 receptor.

[0020] Partial cDNA sequences of IIP-1 have been reported previously. Two overlapping cDNA clones (Fig. 4) were identified which show high homology to the human TIP-2 partial cDNA (GenBank accession number: AF028824) (Rousset, R., et al., Oncogene 16 (1998) 643-654) and were designated as IIP-1a and IIP-1b. The IIP-1a cDNA corresponds to nt 117 to 751 of TIP-2. The IIP-1b cDNA shows besides TIP-2 sequences (nt 1 to 106) additional 5' sequences which are homologous to sequence Y2H35 of the patent WO 97/27296 (nt 25 to 158).

[0021] IIP-1a and IIP-1b both share the sequence coding for the PDZ domain of TIP-2 (nt 156 to 410) which is a known protein-protein interaction domain (Ponting, C.P., et al., BioEssays 19 (1997) 469-479). By deletion analysis the PDZ domain was determined as the essential and sufficient IGF-1 receptor binding domain of IIP-1 (Fig. 4).

[0022] Further yeast two-hybrid analysis revealed that binding of the IIP-1 protein to the IGF-1 receptor is specific for this receptor tyrosine kinase. No interaction was seen to the insulin receptor or Ros. Receptor tyrosine kinases of other families did not interact with IIP-1 (e.g. Met, Ret, Kit, Fms, Neu, EGF receptor). Thus, IIP-1 most likely is the first interaction protein shown to be specific for the IGF-1 receptor tyrosine kinase. IIP-1 also binds to the kinase inactive mutant of the IGF-1 receptor.

IIP-2

[0023] IIP-2 was identified as a new binder of the cytoplasmic part of the IGF-1 receptor which corresponds to human APS (EMBL accession number: HSAB520). APS has been previously isolated in a yeast two-hybrid screen using the oncogenic c-kit kinase domain as bait (Yokouchi, M., et al., Oncogene 15 (1998) 7-15). IIP-2 interacts with the IGF-1 receptor in a kinase dependent manner. Binding of IIP-2 was also observed to other members of the insulin receptor family (insulin receptor, Ros), but not to an unrelated receptor tyrosine kinase (Met). The region of IIP-2 which was found to interact with the IGF-1 receptor (nt 1126 to 1674) contains the SH2 domain of APS (nt 1249 to 1545).

IIP-3

[0024] IIP-3 was isolated as a new IGF-1 receptor interacting protein and is identical to PSM (GenBank accession number: AF020526). PSM is known as a PH and SH2 domain containing signal transduction protein which binds to the activated insulin receptor (Riedel, H., et al., J. Biochem. 122 (1997) 1105-1113). A variant of PSM has also been described (Riedel, H., et al., J. Biochem. 122 (1997) 1105-1113). Binding of IIP-3 to the IGF-1 receptor is dependent on tyrosyl phosphorylation of the receptor.

[0025] A cDNA clone corresponding to nt 1862 to 2184 of the variant form of PSM was identified. The isolated cDNA clone turned out to code for the IGF-1 receptor binding region. The SH2 domain of PSM (nt 1864 to 2148) is encoded by the sequence of the IIP-3 partial cDNA clone isolated.

IIP-4

[0026] IIP-4 was isolated as a new interacting protein of the cytoplasmic domain of the IGF-1 receptor. IIP-4 corresponds to p59fyn, a src-like tyrosine kinase (EMBL accession number: MMU70324 and human fyn EM_HUM1:HS66H14) (Cooke, M.P., and Perlmutter, R.M., New Biol. 1 (1989) 66-74). IIP-4 binds in a kinase dependent manner to the IGF-1 receptor and to several other receptor tyrosine kinases as to the insulin receptor and Met. The region of IIP-4 interacting with the IGF-1 receptor (nt 665 to 1044) contains the SH2 domain of p59fyn (nt).

IIP-5

[0027] IIP-5 was isolated as a new IGF-1 receptor interacting protein. IIP-5 shows a high homology to the zinc finger protein Zfp38 (EMBL accession number: MMZFPTA) and is at least 80% homologous to the corresponding human gene. Zfp-38 is known as a transcription factor (Chowdhury, K., et al., Mech. Dev. 39 (1992) 129-142). IIP-5 interacts exclusively with the activated and phosphorylated IGF-1 receptor, but not with a kinase inactive mutant. In addition to binding of IIP-5 to the IGF-1 receptor interaction of IIP-5 with receptor tyrosine kinases of the insulin receptor family (insulin receptor, Ros) was observed. IIP-5 does not bind to the more distantly related receptor tyrosine kinase Met.

[0028] One cDNA clone binding to the IGF-1 receptor which codes for nt 756 to 1194 of Zfp38 and contains the first zinc finger (nt 1075 to 1158) was isolated. This domain is sufficient for binding to the activated IGF-1 receptor.

IIP-6

[0029] IIP-6 was identified as a new IGF-1 receptor interacting protein. IIP-6 shows weak similarity to the zinc finger domain of Zfp29 (EMBL accession number: MMZFP29). Zfp29 consists of a N-terminal transcriptional activation domain and 14 C-terminal Cys^2His^2 zinc fingers (Denny, P., and Ashworth, A., Gene 106 (1991) 221-227). Binding of IIP-6 to the IGF-1 receptor depends on phosphorylation of the IGF-1 receptor kinase. IIP-6 also binds to the insulin receptor, but does not interact with Met. The region of IIP-6 found to interact with the IGF-1 receptor (SEQ ID NO:3, SEQ ID NO:4) contains two zinc finger domains of the Cys^2His^2 type.

IIP-7

[0030] IIP-7 was isolated as a new IGF-1 receptor interacting protein which corresponds to Pax-3 (EMBL accession number: MMPAX3R and human Pax3 EM-HUM2:S69369). Pax-3 is known as a DNA-binding protein being expressed during early embryogenesis (Goulding, M.D., et al., EMBO J. 10 (1991) 1135-1147). IIP-7 binds in a phosphorylation dependent manner to the IGF-1 receptor. IIP-7 also interacts with the insulin receptor and Met. A partial IIP-7 cDNA clone turned out to code for the IGF-1 receptor binding domain (nt 815 to 1199). This region contains the Pax-3 paired damain octapeptide (nt 853 to 876) and the paired-type homeodomain (nt 952 to 1134).

IIP-8

[0031] IIP-8 was isolated as a new interacting binder of the IGF-1 receptor. IIP-8 is identical to Grb7 (EMBL accession number: MUSGRB7P and human Grb7 EM_HUM1:AB008789). Grb7, a PH domain and a SH3 domain containg signal transduction protein, was first published as an EGF receptor-binding protein (Margolis, B.L., et al., Proc. Natl. Acad. Sci. USA 89 (1992) 8894-8898). IIP-8 does not interact with the kinase inactive mutant of the IGF-1 receptor. Binding of IIP-8 to several other receptor tyrosine kinases (e.g. insulin receptor, Ros and Met) was also observed.

IIP-9

[0032] IIP-9 was identified as a new IGF-1 receptor interaction protein. IIP-9 is identical to nck (EMBL accession number: HSNCK). Nck is a cytoplasmic signal transduction protein consisting of SH2 and SH3 domains (Lehmann, J.M., et al., Nucleic Acids Res. 18 (1990) 1048). IIP-9 interacts with the IGF-1 receptor in a phosphorylation dependent manner. nck binds to the juxtamembrane region of the IGF-1 receptor. Apart from binding of IIP-9 to the IGF-1 receptor, interaction with the insulin receptor but not with Ros or Met was seen.

IIP-10

[0033] IIP-10 was isolated as a new IGF-1 receptor interacting protein. The nucleotide sequence of a partial cDNA clone coding for the IGF-1 receptor binding domain is shown in SEQ ID NO:5. IIP-10 shows 64.4% homology to the Gallus Gallus thymocyte protein thy28kD (EMBL Accession Number: GG34350). IIP-10 interacts with the IGF-1 receptor in a phosphorylation-dependent manner. No interaction with the insulin receptor, Ros or Met was seen.

[0034] A preferred object of the invention are polypeptides that are substantially identical to the polypeptides of SEQ ID NO:2 (IIP-1). By „substantially identical" is meant an amino acid sequence which differs only by conservative amino acid substitutions, for example substitutions of one amino acid for another of the same class (e.g. valine for glycine, arginine for lysine, etc.) or by one or more non-conservative amino acid substitution, deletions or insertions located at positions of the amino acid sequence which do not destroy the biological function of the polypeptide. This includes substitution of alternative covalent peptide bonds in the polypeptide. By „polypeptide" is meant any chain of amino acids regardless of length or posttranslational modification (e.g., glycosylation or phosphorylation) and can be

used interchangeably with the term „protein".

[0035] According to the invention by „biologically active fragment" is meant a fragment which can exert a physiological effect of the full-length naturally-occurring protein (e.g., binding to its biological substrate, causing an antigenic response, etc.).

[0036] The invention also features fragments of the polypeptide according to the invention which are antigenic. The term „antigenic" as used herein refers to fragments of the protein which can induce a specific immunogenic response, e.g. an immunogenic response which yields antibodies which specifically bind to the protein according to the invention. The fragments are preferably at least 8 amino acids, and preferably up to 25 amino acids, in length. In one preferred embodiment, the fragments include the domain which is responsible for the binding of the IIPs to the IGF-1 receptor (i.e., the PDZ domain of IIP-1. By „domain" is meant the region of amino acids in a protein directly involved in the interaction with its binding partner. PDZ domains are approximately 90-residue repeats found in a number of proteins implicated in ion-, channel and receptor clustering and the linking of receptors to effector enzymes. Such PDZ are described in general by Cabral, J.H., et al., Nature 382 (1996) 649-652.

[0037] The invention further comprises a method for producing a protein according to the invention whose expression or activation is correlated with tumor proliferation, by expressing an exogenous DNA in prokaryotic or eukaryotic host cells and isolation of the desired protein or expressing said exogeneous DNA in vivo for pharmaceutical means, wherein the protein is coded preferably by a DNA sequence coding for IIP-1, preferably the DNA sequence shown in SEQ ID NO:1.

[0038] The polypeptides according to the invention can also be produced by recombinant means, or synthetically. Non-glycosylated IIP polypeptide is obtained when it is produced recombinantly in prokaryotes. With the aid of the nucleic acid sequences provided by the invention it is possible to search for the IIP gene or its variants in genomes of any desired cells (e.g. apart from human cells, also in cells of other mammals), to identify these and to isolate the desired gene coding for the IIP protein. Such processes and suitable hybridization conditions are known to a person skilled in the art and are described, for example, by Sambrook et al., Molecular Cloning: A laboratory manual (1989) Cold Spring Harbor Laboratory Press, New York, USA, and Hames, B.D., Higgins, S.G., Nucleic acid hybridisation - a practical approach (1985) IRL Press, Oxford, England. In this case the standard protocols described in these publications are usually used for the experiments.

[0039] The use of recombinant DNA technology enables the production of numerous active IIP derivatives. Such derivatives can, for example, be modified in individual or several amino acids by substitution) deletion or addition. The derivatization can, for example, be carried out by means of site directed mutagenesis. Such variations can be easily carried out by a person skilled in the art (J. Sambrook, B.D. Hames, loc. cit.). It merely has to be ensured by means of the below-mentioned tumor cell growth inhibition assay that the characteristic properties of IIP are preserved. The invention therefore in addition concerns a IIP polypeptide which is a product of prokaryotic or eukaryotic expression of an exogenous DNA.

[0040] With the aid of such nucleic acids coding for an IIP protein, the protein according to the invention can be obtained in a reproducible manner and in large amounts. For expression in prokaryotic or eukaryotic organisms, such as prokaryotic host cells or eukaryotic host cells, the nucleic acid is integrated into suitable expression vectors, according to methods familiar to a person skilled in the art. Such an expression vector preferably contains a regulatable/inducible promoter. These recombinant vectors are then introduced for the expression into suitable host cells such as, e.g., E. coli as a prokaryotic host cell or Saccharomyces cerevisiae, teratocarcinoma cell line PA-1 sc 9117 (Büttner et al., Mol. Cell. Biol. 11 (1991) 3573-3583), insect cells, CHO or COS cells as eukaryotic host cells and the transformed or transduced host cells are cultured under conditions which allow expression of the heterologous gene. The isolation of the protein can be carried out according to known methods from the host cell or from the culture supernatant of the host cell. Such methods are described for example by Ausubel I., Frederick M., Current Protocols in Mol. Biol. (1992), John Wiley and Sons, New York. Also in vitro reactivation of the protein may be necessary if it is not found in soluble form in the cell culture.

[0041] The protein can be isolated from the cells or the culture supernatant and purified by chromatographic means, preferably by ion exchange chromatography, affinity chromatography and/or reverse phase HPLC.

[0042] IIP can be purified after recombinant production by affinity chromatography using known protein purification techniques, including immunoprecipitation, gel filtration, ion exchange chromatography, chromatofocussing, isoelectric focussing, selective precipitation, electrophoresis, or the like.

[0043] The invention further comprises a method for detecting a nucleic acid molecule of an IIP-gene, comprising incubating a sample (e.g., body fluids such as blood, cell lysates) with the isolated nucleic acid molecule according to the invention and determining hybridization under stringent conditions of said isolated nucleic acid molecule to a target nucleic acid molecule for determination of presence of a nucleic acid molecule which is said IIP gene and therefore a method for the identification of IGF-1R activation or inhibition in mammalian cells or body fluids.

[0044] On the basis of the nucleic acids provided by the invention it is possible to provide a test which can be used to detect IIP nucleic acids. Such a test can be carried out by means of nucleic acid diagnostics. In the case of IIP-1 the

sample to be examined is contacted with a probe that is selected from the group comprising

a) the nucleic acids shown in SEQ ID NO:1 or a nucleic acid sequence which is complementary thereto, or
b) nucleic acids which hybridize under stringent conditions with one of the nucleic acids from a), wherein the nucleic acid probe is incubated with the nucleic acid of the sample and the hybridization is detected optionally by means of a further binding partner for the nucleic acid of the sample and/or the nucleic acid probe.

[0045] Probes of a corresponding design are used for the other IIPs according to the invention.

[0046] Hybridization between the probe and nucleic acids from the sample indicates the presence of the RNA of such proteins. Such methods are known to a person skilled in the art and are described, for example, in WO 89/06698, EP-A 0 200 362, USP 2915082, EP-A 0 063 879, EP-A 0 173 251, EP-A 0 128 018.

[0047] In a preferred embodiment of the invention the coding nucleic acid of the sample is amplified before the test, for example by means of the known PCR technique. Usually a derivatized (labeled) nucleic acid probe is used within the framework of nucleic acid diagnostics. This probe is contacted with a denatured DNA or RNA from the sample which is bound to a carrier and in this process the temperature, ionic strength, pH and other buffer conditions are selected - depending on the length and composition of the nucleic acid probe and the resulting melting temperature of the expected hybrid - such that the labeled DNA or RNA can bind to homologous DNA or RNA (hybridization see also Wahl, G.M., et al., Proc. Natl. Acad. Sci. USA 76 (1979) 3683-3687). Suitable carriers are membranes or carrier materials based on nitrocellulose (e.g., Schleicher and Schüll, BA 85, Amersham Hybond, C.), strengthened or bound nitrocellulose in powder form or nylon membranes derivatized with various functional groups (e.g., nitro groups) (e.g., Schleicher and Schüll, Nytran; NEN, Gene Screen; Amersham Hybond M.; Pall Biodyne).

[0048] Hybridizing DNA or RNA is then detected by incubating the carrier with an antibody or antibody fragment after thorough washing and saturation to prevent unspecific binding. The antibody or the antibody fragment is directed towards the substance incorporated during derivatization into the nucleic acid probe. The antibody is in turn labeled. However, it is also possible to use a directly labeled DNA. After incubation with the antibodies it is washed again in order to only detect specifically bound antibody conjugates. The determination is then carried out according to known methods by means of the label on the antibody or the antibody fragment.

[0049] The detection of the expression can be carried out for example as:

- in situ hybridization with fixed whole cells, with fixed tissue smears and isolated metaphase chromosomes,
- colony hybridization (cells) and plaque hybridization (phages and viruses),
- Southern hybridization (DNA detection),
- Northern hybridization (RNA detection),
- serum analysis (e.g., cell type analysis of cells in the serum by slot-blot analysis),
- after amplification (e.g., PCR technique).

[0050] Therefore the invention also includes a method for the detection of the proliferation potential of a tumor cell comprising

a) incubating a sample of body fluid of a patient suffering from cancer, of cancer cells, or of a cell extract or a cell culture supernatant of said cancer cells, whereby said sample contains nucleic acids with a nucleic acid probe which in the case of IIP-1 is selected from the group consisting of

(i) the nucleic acid shown in SEQ ID NO:1, the nucleic acids coding for IIP-2, IIP-3, IIP-4, IIP-5, IIP-6, IIP-7, IIP-8, IIP-9, or IIP-10, or a nucleic acid which is complementary thereto and
(ii) nucleic acids which hybridize under stringent conditions with one of the nucleic acids from (i) and

b) detecting hybridization by means of a further binding partner of the nucleic acid of the sample and/or the nucleic acid probe or by X-ray radiography.

[0051] Preferably the nucleic acid probe is incubated with the nucleic acid of the sample and the hybridization is detected optionally by means of a further binding partner for the nucleic acid of the sample and/or the nucleic acid probe.

[0052] The nucleic acids according to the invention are hence valuable prognostic markers in the diagnosis of the metastatic and progression potential of tumor cells of a patient.

[0053] According to the invention antagonists of IIP or inhibitors for the expression of IPP (e.g., antisense nucleic acids) can be used to inhibit tumor progression and cause massive apoptosis of tumor cells in vivo, preferably by somatic gene therapy.

[0054] Therefore, the present invention also relates to methods of screening for potential therapeutics for cancer, diabetes, neurodegenerative disorders, bone diseases, to methods of treatment for disease and to cell lines and animal models useful in screening for and evaluating potentially useful therapies for such disease. Therefore another object of the invention are methods for identifying compounds which have utility in the treatment of the afore-mentioned and related disorders. These methods include methods for modulating the expression of the polypeptides according to the invention, methods for identifying compounds which can selectively bind to the proteins according to the invention and methods of identifying compounds which can modulate the activity of said polypeptides. These methods may be conducted in vitro and in vivo and may employ the transformed cell lines and transgenic animal models of the invention.

Screening for agonists of IIPs or inhibitors

[0055] An antagonist of IIPs or an inhibitor of IIPs is defined as a substance or compound which inhibits the interaction between IGF-1R and IIP, preferably IIP-1. Therefore the biological activity of IGF-1R decreases in the presence of such a compound. In general, screening procedures for IIP antagonists involve contacting candidate substances with IIP-bearing host cells under conditions favorable for binding and measuring the extent of decreasing receptor mediated signaling (in the case of an antagonist). Such an antagonist is useful as a pharmaceutical agent for use in tumor therapy. For the treatment of diabetes, neural diseases, or bone diseases, stimulation of the signaling pathway is required, i.e., screening for agonists is useful.

[0056] IIP activation may be measured in several ways. Typically, the activation is apparent by a change in cell physiology such as an increase or decrease in growth rate or by a change in the differentiation state or by a change in cell metabolism which can be detected in standard cell assays, for example MTT or XTT assays (Boehringer Mannheim GmbH, DE).

[0057] The nucleic acids and proteins according to the invention could therefore also be used to identify and design drugs which interfere with the interaction of IGF-1R and IIPs. For instance, a drug that interacts with one of the proteins could preferentially bind it instead of allowing binding its natural counterpart. Any drug could bind to the IGF-1 receptor and, thereby, prevent binding of an IIP or, vice versa, bind to an IIP and, thereby, prevent binding of the IGF-1 receptor. In both cases, signal transduction of the IGF-1 receptor system would be modulated (preferably inhibited). Screening drugs for this facility would be relatively easy by establishing a competitive assay (assay standard in the art) between the test compound and interaction of IIPs and the IGF-1 receptor and using purified protein or fragments with the same properties as the binding partners.

[0058] The protein according to the invention is suitable for use in an assay procedure for the identification of compounds which modulate the activity of the proteins according to the invention. Modulating the activity as described herein includes the inhibition or activation of the protein and includes directly or indirectly affecting the normal regulation of said protein activity. Compounds which modulate the protein activity include agonists, antagonists and compounds which directly or indirectly affect the regulation of the activity of the protein according to the invention. The protein according to the invention may be obtained from both native and recombinant sources for use in an assay procedure to identify modulators. In general) an assay procedure to identify modulators will contain the IGF receptor, a protein of the present invention, and a test compound or sample which contains a putative modulator of said protein activity. The test compounds or samples may be tested directly on, for example, purified protein of the invention, whether native or recombinant, subcellular fractions of cells producing said protein, whether native or recombinant, and/or whole cells expressing said protein, whether native or recombinant. The test compound or sample may be added to the protein according to the invention in the presence or absence of known modulators of said protein. The modulating activity of the test compound or sample may be determined by, for example, analyzing the ability of the test compound or sample to bind to said protein, activate said protein, inhibit its activity, inhibit or enhance the binding of other compounds to said protein, modifying receptor regulation or modifying intracellular activity.

[0059] The identification of modulators of the protein activity are useful in treating disease states involving the protein activity. Other compounds may be useful for stimulating or inhibiting the activity of the protein according to the invention. Such compounds could be of use in the treatment of diseases in which activation or inactivation of the protein according to the invention results in either cellular proliferation, cell death, non-proliferation, induction of cellular neoplastic transformations, or metastatic tumor growth and hence could be used in the prevention and/or treatment of cancers such as, for example, prostate and breast cancer. The isolation and purification of a DNA molecule encoding the protein according to the invention would be useful for establishing the tissue distribution of said protein as well as establishing a process for identifying compounds which modulate the activity of said protein and/or its expression.

[0060] Therefore a further embodiment of the invention is a method for screening a compound that inhibits the interaction between IGF-1R and IIP (IIP-1, IIP-2, IIP-3, IIP-4, IIP-5, IIP-6, IIP-7, IIP-8, IIP-9, IIP-10) comprising

a) combining IGF-1R and IIP polypeptide, preferably IIP-1 polypeptide, with a solution containing a candidate compound such that the IGF-1R and IIP polypeptide are capable of forming a complex and

b) determining the amount of complex relative to the predetermined level of binding in the absence of said candidate compound and therefrom evaluating the ability of said candidate compound to inhibit binding of IGF-1R to IIP.

[0061] Such a screening assay is preferably performed as an ELISA assay whereby IGF-1R or IIP is bound on a solid phase.

[0062] A further embodiment of the invention is a method for the production of a therapeutic agent for the treatment of carcinomas in a patient comprising combining a therapeutically effective amount of a substance which inhibits the interaction between IGF-1R and IIP in biochemical and/or cellular assays to an extent of at least 50%. Biochemical assays are preferably ELISA-based assays or homogeneous assays. In the case of the ELISA system antibodies specific for the two binding partners are used for detection of the complexes. In the case of the homogenous assay at least one binding partner is labeled with fluorophores which allows analysis of the complexes. Cellular assays are preferably assays whereby tumor cells or cells transfected with expression constructs of the IGF-1R and the respective binding proteins are treated with or without drugs and complex formation between the two components is then analyzed using standard cell assays.

[0063] A preferred embodiment of the invention is a method for the production of a therapeutic agent for the treatment of carcinomas in a patient comprising combining a pharmaceutically acceptable carrier with a therapeutically effective amount of a substance which inhibits the interaction between IGF-1R and IIP-1, IIP-2, IIP-3, IIP-4, IIP-5, IIP-6, IIP-7, IIP-8, IIP-9, or IIP-10 in a cellular assay, whereby in said cellular assay tumor cells or cells transfected with expression constructs of IGF-1R and of the respective IIP are treated with said substance, and complex formation between IGF-1R and said respective IIP is analyzed, and the extent of said complex formation in the case of inhibition does not exceed 50% referred to 100% for complex formation without said substance in said same cellular assay.

[0064] A further embodiment of the invention is a method of treating a patient suffering from a carcinoma with a therapeutically effective amount of a substance which inhibits the interaction between IGF-1R and IIP-1, IIP-2, IIP-3, IIP-4, IIP-5, IIP-6, IIP-7, IIP-8, IIP-9, or IIP-10 in a cellular assay, whereby in said cellular assay tumor cells or cells transfected with expression constructs of IGF-1R and of the respective IIP are treated with said substance, and complex formation between IGF-1R and said respective IIP is analyzed, and the extent of said complex formation in the case of inhibition does not exceed 50% referred to 100% for complex formation without said substance in said same cellular assay.

[0065] A further embodiment of the invention is an antibody against the IIPs according to the invention.

[0066] Antibodies were generated from the human, mouse, or rat polypeptides. Antibodies specifically recognizing IIP are encompassed by the invention. Such antibodies are raised using standard immunological techniques. Antibodies may be polyclonal or monoclonal or may be produced recombinantly such as for a humanized antibody. An antibody fragment which retains the ability to interact with IIP is also provided. Such a fragment can be produced by proteolytic cleavage of a full-length antibody or produced by recombinant DNA procedures. Antibodies of the invention are useful in diagnostic and therapeutic applications. They are used to detect and quantitate IIP in biological samples, particularly tissue samples and body fluids. They are also used to modulate the activity of IIP by acting as an agonist or an antagonist.

[0067] The following examples, references, sequence listing and drawing are provided to aid the understanding of the present invention, the true scope of which is set forth in the appended claims. It is understood that modifications can be made in the procedures set forth without departing from the spirit of the invention.

Description of the Figures and Sequences

[0068]

Figure 1 Domain structure of yeast two-hybrid baits which were used to screen cDNA libraries for cytoplasmic binding proteins of the IGF-1 receptor. The LexA DNA binding domain was fused to the cytoplasmic (cp) domain (nt 2923 to 4154) of the wildtype IGF-1 receptor (a) or the kinase inactive mutant (K/A mutation at aa 1003) (b) (Ullrich, A., et al., EMBO J. 5 (1986) 2503-2512; Weidner, M., et al., Nature 384 (1996) 173-176). The nucleotide and amino acid sequence of two different linkers inserted between the LexA DNA-binding domain and the receptor domain are shown below. The I1 (wt IGF-1 receptor) and K1 (kinase inactive mutant IGF-1 receptor) constructs contain an additional proline and glycine compared to the I2 and K2 constructs.

Figure 2 Modification of the yeast two-hybrid LexA/IGF-1 receptor bait construct.

a) Schematic illustration of cytoplasmic binding sites of the IGF-1 receptor. The α-subunits of the IGF-1 receptor are linked to the β-chains via disulfid bonds. The cytoplasmic part of the β-chain

contains binding sites for substrates in the juxtamembrane and C-terminal domain.

b) Domain structure of the two-hybrid bait containing only the juxtamembrane IGF-1 receptor binding sites. The juxtamembrane domain of the IGF-1 receptor (nt 2923 to 3051) (Ullrich, A., et al., EMBO J. 5 (1986) 2503-2512) was fused to the kinase domain of tprmet (nt 3456 to 4229) (GenBank accession number: HSU19348).

c) Domain structure of the two-hybrid bait containing only the C-terminal IGF-1 receptor binding sites. The C-terminal domain of the IGF-1 receptor (nt 3823 to 4149) (Ullrich, A., et al., EMBO J. 5 (1986) 2503-2512) was fused to the kinase domain of tprmet (nt 3456 to 4229) (GenBank accession number: HSU19348).

Figure 3 Isoforms of IIP-1.

a) Delineation of the cDNA sequences of IIP-1 and IIP-1 (p26). Nucleotides are numbered above. The potential translation initiation site within the IIP-1 cDNA is at position 63. The first ATG as potential translation initiation site in the alternative splice variant IIP-1 (p26) is at position 353. Both cDNAs contain a stop codon at position 1062.

b) Domain structure of IIP-1 and IIP-1 (p26). Amino acid positions are indicated above. In comparison to IIP-1 (p26) IIP-1 contain additional 97 amino acids at the N-terminus. Both isoforms of IIP-1 contain a PDZ domain spanning a region between amino acids 129 and 213.

Figure 4 Delineation of the IGF-1 receptor binding domain of IIP-1.

Full-length IIP-1, its partial cDNA clones (IIP-1a and IIP-1b) and deletion mutants (IIP-1a/mu1, IIP-1a/mu2, IIP-1a/mu3, IIP-1b/mu1) were examined for interaction with the IGF-1 receptor in the yeast two-hybrid system. Yeast cells were cotransfected with a LexA IGF-1 receptor fusion construct and an activation plasmid coding for IIP-1 or the different IIP-1 mutants fused to the VP16 activation domain. Interaction between IIP-1 or its mutants and the IGF-1 receptor was analyzed by monitoring growth of yeast transfectants plated out on histidine deficient medium and incubated for 6d at 30°C (diameter of yeast colonies: +++, > 1 mm in 2d; ++, > 1 mm in 4d; +, > 1 mm in 6d; -, no detected growth). The PDZ domain can be defined as essential and sufficient for mediating the interaction with the IGF-1 receptor. Nucleotide positions with respect to full length IIP-1 are indicated above.

SEQ ID NO:1 Nucleotide sequence of IIP-1.

SEQ ID NO:2 Predicted amino acid sequence of IIP-1.

SEQ ID NO:3 Nucleotide sequence of the IIP-6 partial cDNA clone.

SEQ ID NO:4 Deduced amino acid sequence of the IIP-6 partial cDNA clone. Cysteine and histidine residues of the two Cys_2His_2 Zinc finger domains are amino acids 72, 75, 88, 92, 100, 103, 116, and 120.

SEQ ID NO:5 Nucleotide sequence of the IIP-10 partial cDNA clone.

SEQ ID NO:6 Deduced amino acid sequence of the IIP-10 partial cDNA clone.

SEQ ID NO:7 Primer TIP2c-s.

SEQ ID NO:8 Primer TIP2b-r.

Example 1

Isolation and characterization of IGF-1R binding proteins

[0069] The yeast two-hybrid system (Fields, S., and Song, O., Nature 340 (1989) 245-246) was used to isolate unknown cytosolic IGF-1 receptor binding proteins. For screening a modified version of the yeast two-hybrid system was used which allows interchain tyrosylphosphorylation of the receptors in yeast.

[0070] The yeast two-hybrid bait plasmid (BTM116-cpIGF-1 receptor) was constructed by fusing the cytoplasmic domain of the β-subunit of the IGF-1 receptor (nt 2923 to 4154) (Ullrich, A., et al., EMBO J. 5 (1986) 2503-2512) to the LexA DNA-binding domain which forms dimers and mimics the situation of the activated wildtype receptor (cf. Weidner,

M., et al., Nature 384 (1996) 173-176). By introducing a proline-glycine spacer between the LexA DNA-binding domain and the receptor domain the ability of the bait to bind known substrates of the IGF-1 receptor was remarkably increased in comparision to other spacer amino acids (Fig. 1).

[0071] Alternatively a bait was constructed containing only the juxtamembrane or C-terminal region of the IGF-1 receptor (nt 2923 to 3051 or nt 3823 to 4146) (Ullrich, A., et al., EMBO J. 5 (1986) 2503-2512) fused to the kinase domain of an unrelated, very potential receptor tyrosine kinase. Here the kinase domain of tpr met (nt 3456 to 4229) (GenBank accession number: HSU19348) (Fig. 2) was used. In this way it is possible to delineate the region of the IGF-1 receptor which mediates binding to downstream effectors.

[0072] The IGF-1 receptor bait plasmid was used to screen activation domain cDNA libraries (e.g. VP16- or Gal4 based activation domain) (cf. Weidner, M., et al., Nature 384 (1996) 173-176). The bait and prey plasmids were co-transfected into Saccharomyces cerevisiae strain L40 containing a HIS3 and lacZ reporter gene. Library plasmids were isolated from yeast colonies growing on histidine deficient medium, were sequenced and reintroduced into yeast strain L40. By co-transfecting experiments with different test baits, i.e. BTM116 plasmids coding for a kinase inactive mutant of the IGF-1 receptor (L1033A) or the cytoplasmic domain of receptor tyrosine kinases of the insulin receptor family (insulin receptor, Ros) and of unrelated receptor tyrosine kinase families (Met, ECF receptor, Kit, Fms, Neu) the specificity of the putative bait-prey interactions was evaluated. Several cDNAs were identified which code for previously unknown IGF-1 receptor interacting proteins (IIPs). In addition binding domains of known substrates of the IGF-1 receptor such as the C-terminal SH2 domain of p85PI3K and the SH2 domain of Grb10 were found. The results are shown in Table 1.

Table 1

IIP	wt IGF-1R	mu IGF-1R	IR	Ros	Met
IIP-1	+	+	-	-	-
IIP-2	+	-	+	+	-
IIP-3	+	-	+	+	+
IIP-4	+	-	+	nd	+
IIP-5	+	-	+	+	-
IIP-6	+	-	+	nd	-
IIP-7	+	-	+	nd	+
IIP-8	+	-	+	+	+
IIP-9	+	-	+	-	-
IIP-10	+	-	-	-	-

[0073] Delineation of the binding specificity of the IIPs with respect to different receptor tyrosine kinases tested in the yeast two-hybrid system. Yeast cells were cotransfected with a LexA fusion construct coding for the different receptor tyrosine kinases and an activation plasmid coding for the different IIPs fused to the VP16 activation domain. Interaction between the IIPs and the different receptor tyrosine kinases was analyzed by monitoring growth of yeast transfectants plated out on histidine deficient medium and incubated for 3d at 30°C (wt IGF-1R, kinase active IGF-1 receptor; mu IGF-1 R, kinase inactive mutant IGF-1 receptor; IR, insulin receptor; Ros, Ros receptor tyrosine kinase; Met, Met receptor tyrosine kinase; +, growth of yeast transfectants within 3 days larger than 1mm in diameter; -, no detected growth; nd, not determined).

Example 2

Assay systems:

A) In-vitro/biochemical assays:

[0074]

ELISA-based assays
Homogenous assays

[0075] IGF-1R and the binding proteins (IIPs) are expressed with or without Tag-enzymes in E.coli or eucaryotic cells and purified to homogeneity. Interaction of IGF-1R and the respective binding proteins is analyzed in the presence or absence of drugs. Compounds which either inhibit or promote binding of IGF-1R and the respective binding proteins are selected and further developed. In the case of the ELISA system antibodies specific for the two binding partners are used for detection of the complexes. In the case of the homogenous assay at least one binding partner is labeled with fluorophores which allows analysis of the complexes. Alternatively, anti-Tag-antibodies are used to monitor interaction.

B) Cellular assays:

[0076] Tumor cells or cells transfected with expression constructs of the IGF-1R and the respective binding proteins are treated with or without drugs and complex formation between the two components is then analyzed using standard assays.

Example 3

cDNA cloning of IIP-1 (and RT-PCR-assay)

[0077] The nucleotide sequence of full length IIP-1 was aligned using database information (ESTs) and sequences of the partial cDNA clones of IIP-1 (IIP-1a, IIP-1b). cDNA cloning of full length IIP-1 was performed by RT PCR on total RNA isolated from a $MCF7_{ADR}$breast cell line. PT PCR with two oligonucleotide primers: TIP2c-s (SEQ ID NO:7) and TIP2b-r (SEQ ID NO:8) resulted in amplification of two DNA fragments of 1.0 kb (IIP-1) and 0.7kb (IIP-1(p26)).

[0078] DNA sequencing was performed using the dideoxynucleotide chain termination method on an ABI 373A sequencer using the Ampli Taq® FS Dideoxyterminator kit (Perkin Elmer, Foster City, CA). Comparison of the cDNA and deduced protein sequences was performed using Advanced Blast Search (Altschul, S.F., et al., J. Mol. Biol. 215 (1990) 403-410; Altschul, S.F., et al., Nucleic Acids Res. 25 (1997) 3389-3402).

Example 4

Western blot analysis of IPP- 1

[0079] Total cell lysates were prepared in a buffer containing 50 mM Tris pH 8.0, 150 mM NaCl, 1% NP40, 0.5 % deoxycholic acid, 0.1 % SDS, and 1 mM EDTA pH and cleared by centrifugation for 15 mm at 4° C. The protein concentration of the supernatants was measured using the Micro BCA Protein Assay kit (Pierce Chemical Co., Rockford, IL) according to the manufacturer's manual. IGF-1 receptors were immunoprecipitated using anti-IGF-1 receptor antibodies (Santa Cruz). Proteins were fractionated by SDS-PAGE and electrophoretically transferred to nitrocellulose filters. Nitrocellulose filters were preincubated with 10 % (w/v) fat-free milk powder in 20 mM Tris pH 7.5, 150 mM NaCl, 0.2% Tween-20. Binding of a mouse monoclonal antibody directed against the flag epitope was detected by horseradish peroxidase-labeled goat-anti-mouse IgG antiserum (Biorad, Munich, DE) and visualized using an enhanced chemoluminescence detection system, ECL™ (Amersham, Braunschweig, DE).

Example 5

Overexpression of IIP-1 in LB cells by retrovirus-mediated gene transfer

[0080] NIH 3T3 cells or other recipient cells were transfected with pBAT flag-IIP-1 and pSV2neo and cell clones were selected in 1-10 mg/ml G418 (cf. Weidner, M., et al., Nature 384 (1996) 173-176). Single clones were picked and analyzed for expression of IIP-1 and functionally characterized with respect to proliferation.

Northern blot analysis

[0081] Human and murine mRNA multiple tissue Northern blots were purchased from Clontech (Palo Alto, CA, US). A cDNA probe spanning the coding region for the PDZ domain of murine IIP-1 (bp 447 to 701) was labeled with DIG-dUTP using the PCR DIG Labeling Mix (Boehringer Mannnheim GmbH, DE). A digoxygenin labeled actin RNA probe was purchased from Boehringer Mannheim GmbH, DE. Hybridization was performed using the DIG EasyHyb hybridization solution (Boehringer Mannheim GmbH, DE). IIP-1 mRNA was detected with DIG-specific antibodies conjugated to alkaline phosphatase and the CSPD substrate (Boehringer Mannheim GmbH, DE).

Example 6

Detection of mRNA in cancer cells

[0082] In order to detect whether proteins are expressed in cancer cells which are coded by nucleic acids which hybridize with SEQ ID NO:1 and consequently whether mRNA is present, it is possible on the one hand to carry out the established methods of nucleic acid hybridization such as Northern hybridization, in-situ hybridization, dot or slot hybridization and diagnostic techniques derived therefrom (Sambrook et al., Molecular Cloning: A laboratory manual (1989) Cold Spring Harbor Laboratory Press, New York, USA; Hames, B.D., Higgins, S.G., Nucleic acid hybridisation - a practical approach (1985) LRL Press, Oxford, England; WO 89/06698; EP-A 0 200 362; USP 2915082; EP-A 0 063 879; EP-A 0 173 251; EP-A 0 128 018). On the other hand it is possible to use methods from the diverse repertoire of amplification techniques using specific primers (PRC Protocols - A Guide to Methods and Applications (1990), publ. M.A. Innis, D.H. Gelfand, J.J. Sninsky, T.J. White, Academic Press Inc.; PCR - A Practical Approach (1991), publ. M.J. McPherson, P. Quirke, G.R. Taylor, IRL Press). The RNA for this is isolated from the cancer tissue by the method of Chomczynski and Sacchi, Anal. Biochem. 162 (1987) 156-159. 20 µg total RNA was separated on a 1.2% agarose formaldehyde gel and transferred onto nylon membranes (Amersham, Braunschweig, DE) by standard methods (Sambrook et al., Molecular Cloning: A laboratory manual (1989) Cold Spring Harbor Laboratory Press, New York, USA. The DNA sequence SEQ ID NO: 1 was radioactively labeled as probes (Feinberg, A.P., and Vogelstein, B., Anal. Biochem. 137 (1984) 266-267). The hybridization was carried out at 68°C in 5 x SSC, 5 x Denhardt, 7% SDS/0.5 M phosphate buffer pH 7.0, 10% dextran sulfate and 100 µg/ml salmon sperm DNA. Subsequently the membranes were washed twice for one hour each time in 1 x SSC at 68°C and then exposed to X-ray film.

Example 7

Procedure for identification of modulators of the activity of the protein according to the invention

[0083] The expression vector of Example 5 is transferred into NIH 3T3 cells by standard methods known in the art (Sambrook et al.). Cells which have taken up the vector are identified by their ability to grow in the presence of the selection or under selective conditions. Cells which express DNA encoding IIP produce RNA which is detected by Northern blot analysis as described in example 5. Alternatively, cells expressing the protein are identified by identification of the protein by Western blot analysis using the antibodies described in example 4. Cells which express the protein from the expression vector will display an altered morphology and/or enhanced growth properties.

[0084] Cells which express the protein and display one or more of the altered properties described above are procultured with and without a putative modulator compound. The modulator compound will cause an increase or a decrease in the cellular response to the IIP protein activity and will be either an activator or an inhibitor of IGF-receptor function, respectively.

[0085] Alternatively, putative modulators are added to cultures of tumor cells, and the cells display an altered morphology and/or display reduced or enhanced growth properties. A putative modulator compound is added to the cells with and without IIP protein and a cellular response is measured by direct observation of morphological characteristics of the cells and/or the cells are monitored for their growth properties. The modulator compound will cause an increase or a decrease in the cellular response to IIP protein and will be either an activator or an inhibitor of IGF-1 receptor activity, respectively.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: IGF-1 receptor interacting proteins (IIPs), genes coding therefor and use thereof

(iii) NUMBER OF SEQUENCES: 8

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30B (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1707 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GAAACCCACA GGAGGCAACC ACACTAGTTT AGATCTTCTG GTGACCCCAC TTCTCGCTGC 60

TCATGCCGCT GGGACTGGGG CGGCGGAAAA AGGCGCCCCC TCTAGTGGAA AATGAGGAGG 120

CTGAGCCAGG CCGTGGAGGG CTGGGCGTGG GGGAGCCAGG GCCTCTGGGC GGAGGTGGGT 180

CGGGGNNCCC CCAAATGGGC TTNCNCCCCC CTCCCCCAGC CCTGCGGCCC CGCCTCGTGT 240

TCCACACCCA GCTGGCCCAT GGCAGTCCCA CTGGCCGCAT CGAGGGCTTC ACCAACGTCA 300

AGGAGCTGTA TGGCAAGATC GCCGAGGCCT TCCGCCTGCC AACTGCCGAG GTGATGTTCT 360

GCACCCTGAA CACCCACAAA GTGGACATGG ACAAGCTCCT GGGGGGCCAG ATCGGGCTGG 420

AGGACTTCAT CTTCGCCCAC GTGAAGGGGC AGCGCAAGGA GGTGGAGGTG TTCAAGTCGG 480

AGGATGCACT CGGGCTCACC ATCACGGACA ACGGGGCTGG CTACGCCTTC ATCAAGCGCA 540

TCAAGGAGGG CAGCGTGATC GACCACATCC ACCTCATCAG CGTGGGCGAC ATGATCGAGG 600

CCATTAACGG GCAGAGCCTG CTGGGCTGCC GGCACTACGA GGTGGCCCGG CTGCTCAAGG 660

AGCTGCCCCG AGGCCGTACC TTCACGCTGA AGCTCACGGA GCCTCGCAAG GCCTTCGACA 720

TGATCAGCCA GCGTTCAGCG GGTGGCCGCC CTGGCTCTGG CCCACAACTG GGCACTGGCC 780

GAGGGACCCT GCGGCTCCGA TCCCGGGGCC CCGCCACGGT GGAGGATCTG CCCTCTGCCT 840

TTGAAGAGAA GGCCATTGAG AAGGTGGATG ACCTGCTGGA GAGTTACATG GGTATCAGGG 900

ACACGGAGCT GGCAGCCACC ATGGTGGAGC TGGGAAAGGA CAAAAGGAAC CCGGATGAGC 960

TGGCCGAGGC CCTGGACGAA CGGCTGGGTG ACTTTGCCTT CCCTGACGAG TTCGTCTTTG 1020

ACGTCTGGGG CGCCATTGGG GACGCCAAGG TCGGCCGCTA CTAGGACTGC CCCCGGACCC 1080

TGCGATGATG ACCCGGGCGC AACCTGGTGG GGGCCCCCAG CAGGGACACT GACGTCAGGA 1140

CCCGAGCCTC CAGCCTGAGC CTAGCTCAGC AGCCCAAGGA CGATGGTGAG GGGAGGTGGG 1200

GCCAGGCCCC CTGCCCCGCT CCACTCGGTA CCATCCCCTC CCTGGTTCCC AGTCTGGCCG 1260

GGGTCCCCGG CCCCCCTGTG CCCTGTTCCC CACCTACCTC AGCTGGGTCA GGCACAGGGA 1320

GGGGAGGGAT CAGCCAAATT GGGCGGCCAC CCCCGCCTCC ACCACTTTCC ACCATCAGCT 1380

GCCAAACTGG TCCCTCTGTC TCCCTGGGGC CTTGGGTTCT GTTTGGGGGT CATGACCTTC 1440

CTAGTTTCCT GACGCAGGGA ATACAGGGGA GAGGGTTGTC CTTCCCCCCA GCAAATGCAA 1500

TAATGCCCTC ACCCCTCCTG AGAGGAGCCC CCTCCCTGTG GAGCCTGTTA CCTCCGCATT 1560

TGACACGAGT CTGCTGTGAA CCCCGCAACC TCCTCCCCAC CTCCCATCTC TCCTTCCAGG 1620

CCCATCCCTG GCCCAGAGCA GGAGGGAGGG AGGGACGATG GCGGTGGGTT TTTGTATCTG 1680

AATTTGCTGT CTTGAACATA AAGAATC 1707

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 333 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Pro Leu Gly Leu Gly Arg Arg Lys Lys Ala Pro Pro Leu Val Glu
1               5                  10                  15

Asn Glu Glu Ala Glu Pro Gly Arg Gly Gly Leu Gly Val Gly Glu Pro
            20                  25                  30

Gly Pro Leu Gly Gly Gly Gly Ser Gly Xaa Pro Gln Met Gly Xaa Xaa
        35                  40                  45

Pro Pro Pro Pro Ala Leu Arg Pro Arg Leu Val Phe His Thr Gln Leu
    50                  55                  60

Ala His Gly Ser Pro Thr Gly Arg Ile Glu Gly Phe Thr Asn Val Lys
65                  70                  75                  80

Glu Leu Tyr Gly Lys Ile Ala Glu Ala Phe Arg Leu Pro Thr Ala Glu
                85                  90                  95

Val Met Phe Cys Thr Leu Asn Thr His Lys Val Asp Met Asp Lys Leu
            100                 105                 110

Leu Gly Gly Gln Ile Gly Leu Glu Asp Phe Ile Phe Ala His Val Lys
        115                 120                 125

Gly Gln Arg Lys Glu Val Glu Val Phe Lys Ser Glu Asp Ala Leu Gly
    130                 135                 140

Leu Thr Ile Thr Asp Asn Gly Ala Gly Tyr Ala Phe Ile Lys Arg Ile
145                 150                 155                 160

Lys Glu Gly Ser Val Ile Asp His Ile His Leu Ile Ser Val Gly Asp
                165                 170                 175

Met Ile Glu Ala Ile Asn Gly Gln Ser Leu Leu Gly Cys Arg His Tyr
            180                 185                 190

Glu Val Ala Arg Leu Leu Lys Glu Leu Pro Arg Gly Arg Thr Phe Thr
        195                 200                 205

Leu Lys Leu Thr Glu Pro Arg Lys Ala Phe Asp Met Ile Ser Gln Arg
    210                 215                 220

Ser Ala Gly Gly Arg Pro Gly Ser Gly Pro Gln Leu Gly Thr Gly Arg
225                 230                 235                 240

Gly Thr Leu Arg Leu Arg Ser Arg Gly Pro Ala Thr Val Glu Asp Leu
                245                 250                 255

Pro Ser Ala Phe Glu Glu Lys Ala Ile Glu Lys Val Asp Asp Leu Leu
            260                 265                 270

Glu Ser Tyr Met Gly Ile Arg Asp Thr Glu Leu Ala Ala Thr Met Val
        275                 280                 285
```

Glu Leu Gly Lys Asp Lys Arg Asn Pro Asp Glu Leu Ala Glu Ala Leu
290 295 300

Asp Glu Arg Leu Gly Asp Phe Ala Phe Pro Asp Glu Phe Val Phe Asp
305 310 315 320

Val Trp Gly Ala Ile Gly Asp Ala Lys Val Gly Arg Tyr
325 330

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 380 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GCCGAGGAAG GAGAAGGGGC TAAACCTTGG AGAGTGGATG GCTCAAAGGA TTCTCAGATC 60

ACACCTCGGG AGGATCATGG GCAGGAGAGC CTGTTGGCAG GGCTCCACGG AACGCATCCA 120

CCAAAGACAA GGCAGAAAGT CACTGCCCAA GCCGGAGGCC CCGGGGATCC CATGCTTTTT 180

TCAAGCCCAG AGACAGATGA GAAGCTTTTT ATATGTGCGC AGTGTGGCAA AACCTTCAAC 240

AATACCTCCA ACCTGAGAAC GCACCAGCGG ATCCACACTG GCGAGAAGCC CTACATGTGT 300

TCCGAGTGTG GCAAGAGTTT CTCCCGGAGC TCCAACCGCA TCCGGCACGA GCGCATCCAC 360

CTGGAAGANA AGCACTCTGA 380

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 126 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ala Glu Glu Gly Glu Gly Ala Lys Pro Trp Arg Val Asp Gly Ser Lys
1 5 10 15

Asp Ser Gln Ile Thr Pro Arg Glu Asp His Gly Gln Glu Ser Leu Leu
20 25 30

Ala Gly Leu His Gly Thr His Pro Pro Lys Thr Arg Gln Lys Val Thr
35 40 45

Ala Gln Ala Gly Gly Pro Gly Asp Pro Met Leu Phe Ser Ser Pro Glu
50 55 60

Thr Asp Glu Lys Leu Phe Ile Cys Ala Gln Cys Gly Lys Thr Phe Asn
65 70 75 80

Asn Thr Ser Asn Leu Arg Thr His Gln Arg Ile His Thr Gly Glu Lys
85 90 95

Pro Tyr Met Cys Ser Glu Cys Gly Lys Ser Phe Ser Arg Ser Ser Asn
100 105 110

Arg Ile Arg His Glu Arg Ile His Leu Glu Xaa Lys His Ser
115 120 125

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 204 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GTGGAGGACT CCAACCCTCA GAAGACTTCA GCCACTAAAA ACTGTTTGAA GAATCTAAGC 60

AGCCACTGGC TGATGAAGTC AGAGCCAGAG AGCCGCCTAG AGAAAGGTGT AGATGTGAAG 120

TTCAGCATTG AGGATCTCAA AGCACAGCCC AAACAGACNA CATGCTGGGA TGGTGTTCGT 180

AACTACCAGG CTCGGAACTT CCTT 204

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 68 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

```
Val Glu Asp Ser Asn Pro Gln Lys Thr Ser Ala Thr Lys Asn Cys Leu
1               5                  10                  15

Lys Asn Leu Ser Ser His Trp Leu Met Lys Ser Glu Pro Glu Ser Arg
            20                  25                  30

Leu Glu Lys Gly Val Asp Val Lys Phe Ser Ile Glu Asp Leu Lys Ala
        35                  40                  45

Gln Pro Lys Gln Thr Thr Cys Trp Asp Gly Val Arg Asn Tyr Gln Ala
    50                  55                  60

Arg Asn Phe Leu
65
```

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Primer TIP2c-s"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GAAACCCACA GGAGGCAA 18

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "Primer TIP2b-r"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GGTCATCATC GCAGGGTC 18

Claims

1. An isolated nucleic acid molecule (IPP-1) coding for a protein binding to the IGF-1 receptor selected from the group

comprising

a) the nucleic acids shown in SEQ ID NO:1 or a nucleic acid sequence which is complementary thereto, or

b) nucleic acids which hybridize under stringent conditions with one of the nucleic acids from a).

2. A recombinant expression vector which is suitable for the expression of a nucleic acid molecule as claimed in claim 1.

3. A recombinant polypeptide which binds to the IGF-1 receptor and is coded by the nucleic acid sequence shown in SEQ ID NO:1 or a nucleic acid which hybridizes under stringent conditions with a nucleic acid complementary to the nucleic acid shown in SEQ ID NO:1.

4. A method for the production of a protein which binds to the IGF-1 receptor, by expressing an exogenous DNA in prokaryotic or eukaryotic host cells and isolation of the desired protein, wherein the protein is coded by the DNA sequence shown in SEQ ID NO:1 or a nucleic acid which hybridizes under stringent conditions with a nucleic acid complementary to the nucleic acid shown in SEQ ID NO:1.

5. A method for the detection of the proliferation potential of a cancer cell comprising

a) incubating a sample of body fluid of a patient suffering from cancer, of tumor cells, or of a cell extract or a cell culture supernatant of said tumor cells, whereby said sample contains nucleic acids with a nucleic acid probe which is selected from the group consisting of

(i) the nucleic acid shown in SEQ ID NO:1, the nucleic acids coding for IIP-2, IIP-3, IIP-4, IIP-5, IIP-6, IIP-7, IIP-8, IIP-9, or IIP-10 or a nucleic acid which is complementary thereto and
(ii) nucleic acids which hybridize with one of the nucleic acids from (i) and

b) detecting the hybridization by means of a further binding partner of the nucleic acid of the sample and/or the nucleic acid probe.

6. The method of claim 5, wherein hybridization is effected at least with the nucleic acid fragment of SEQ ID NO:1 or the complementary fragment.

7. The method of claim 5 or 6, wherein the nucleic acid to be detected is amplified before the detection.

8. A method for screening a compound that inhibits the interaction between IGF-1R and IIP-1, IIP-2, IIP-3, IIP-4, IIP-5, IIP-6, IIP-7, IIP-8, IIP-9, or IIP-10 comprising

a) combining IGF-1R and said IIP polypeptide with a solution containing a candidate compound such that the IGF-1R and said IIP polypeptide are capable of forming a complex and
b) determining the amount of complex relative to the predetermined level of binding in the absence of the compound and therefrom evaluating the ability of the compound to inhibit binding of IGF-1R to said IIP.

9. A method for the production of a therapeutic agent for the treatment of carcinomas in a patient comprising combining a pharmaceutically acceptable carrier with a therapeutically effective amount of a substance which modulates the interaction between IGF-1R and IIP-1, IIP-2, IIP-3, IIP-4, IIP-5, IIP-6, IIP-7, IIP-8, IIP-9, or IIP-10 in a cellular assay, whereby in said cellular assay tumor cells or cells transfected with expression constructs of IGF-1R and of the respective IIP are treated with said substance, and complex formation between IGF-1R and said respective IIP is analyzed, and the extent of said complex formation in the case of inhibition does not exceed 50% referred to 100% for complex formation without said substance in said same cellular assay.

10. A method according to claim 9, wherein the substance inhibits the interaction.

Fig. 1

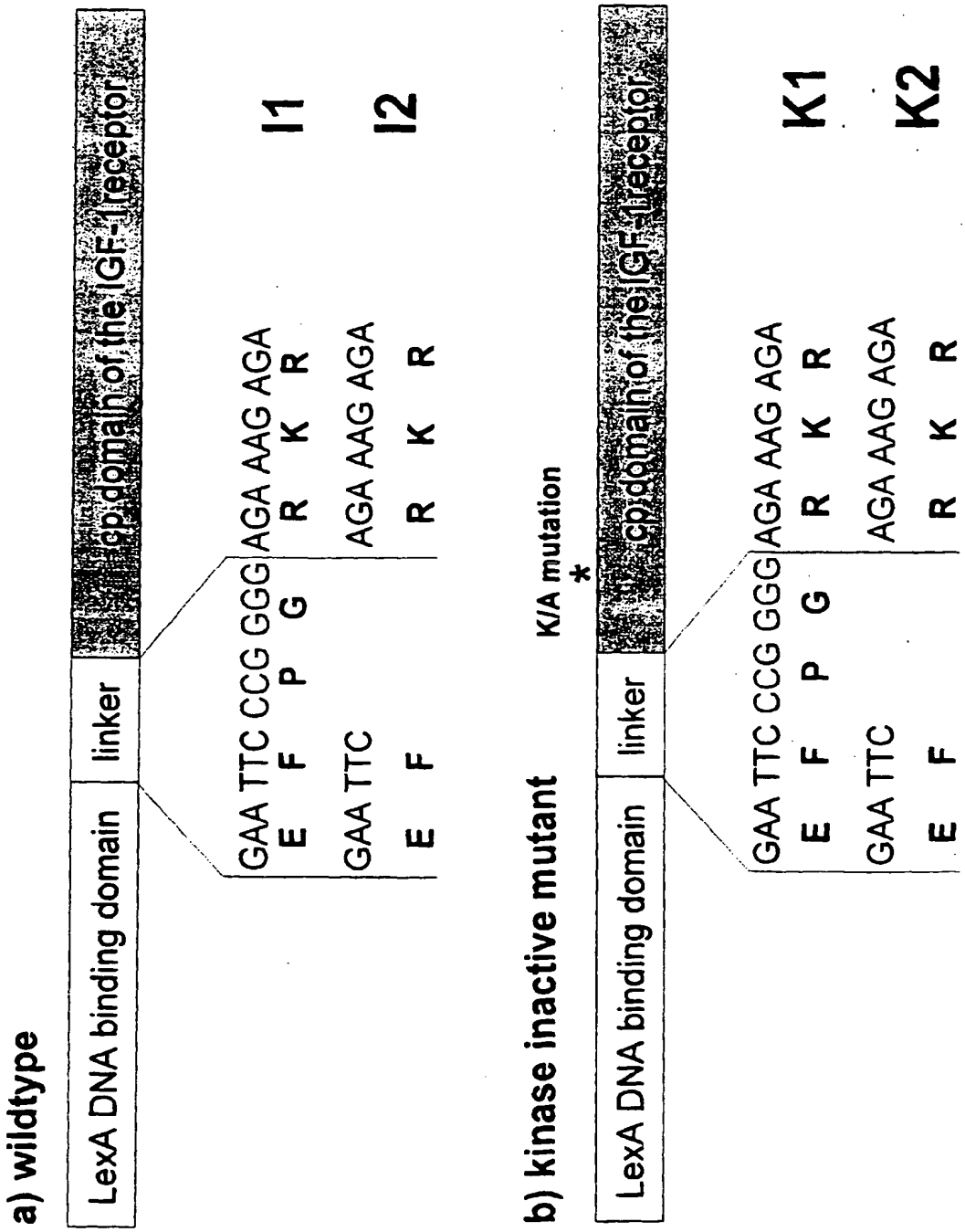


Fig. 2

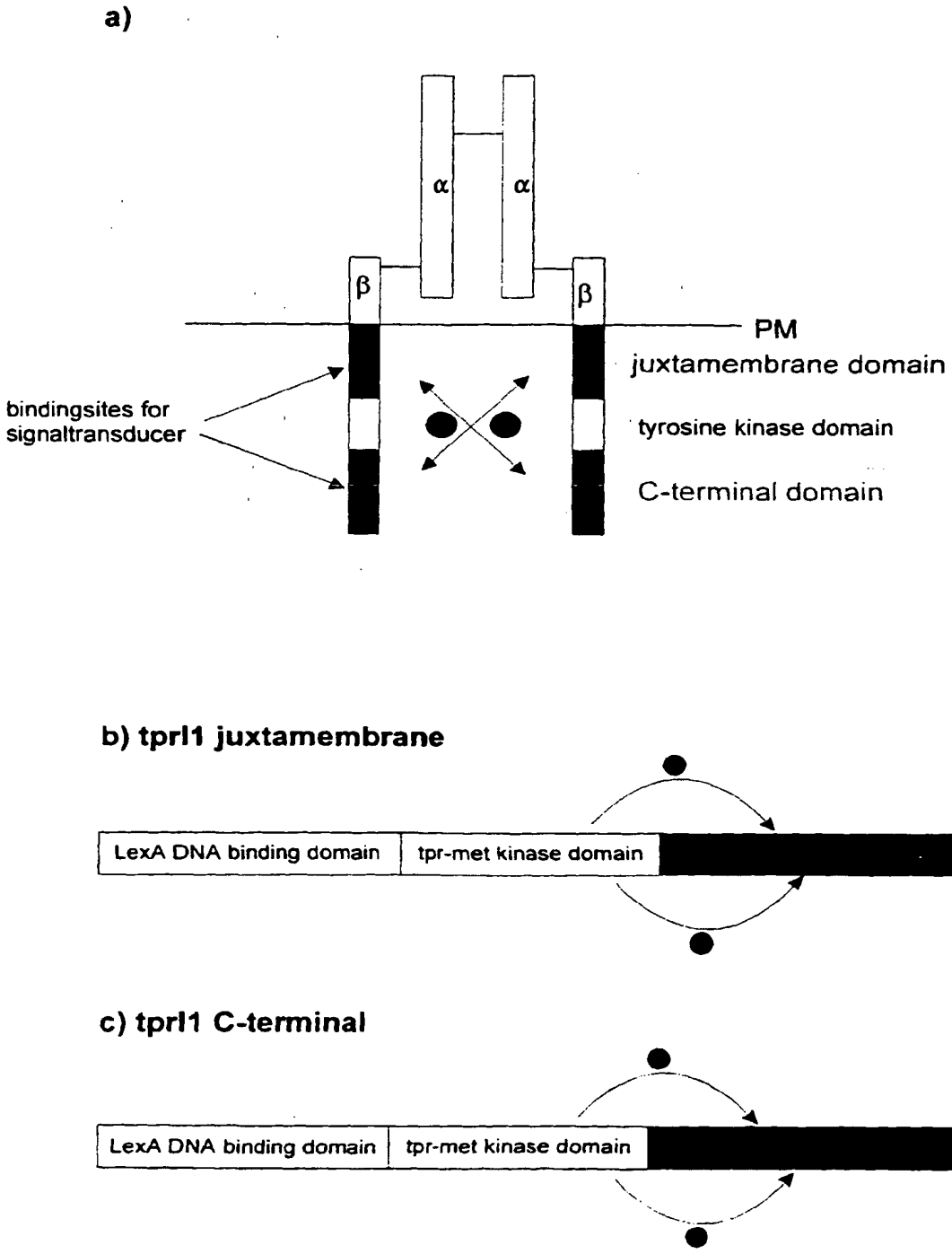


Fig. 3

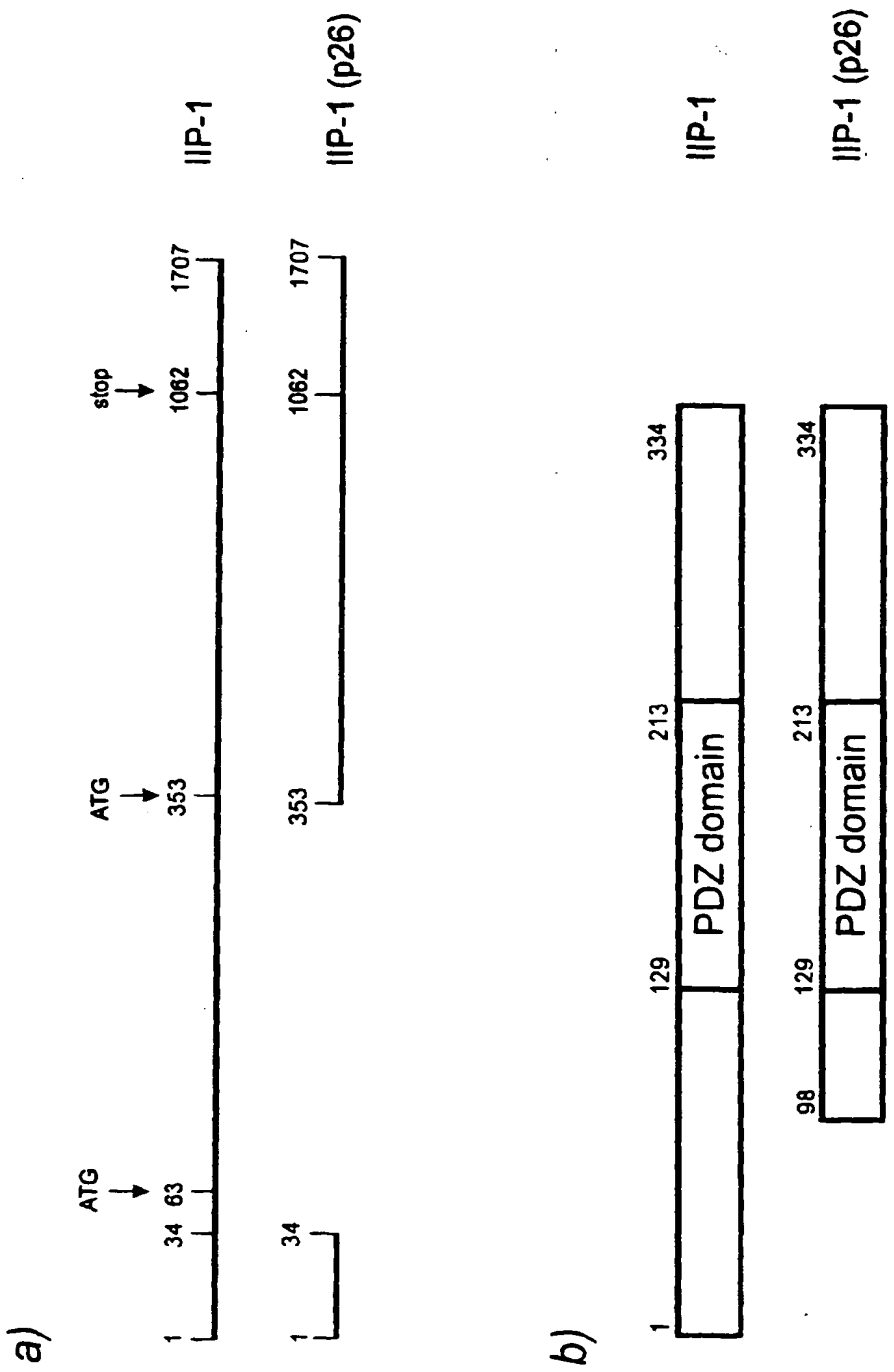
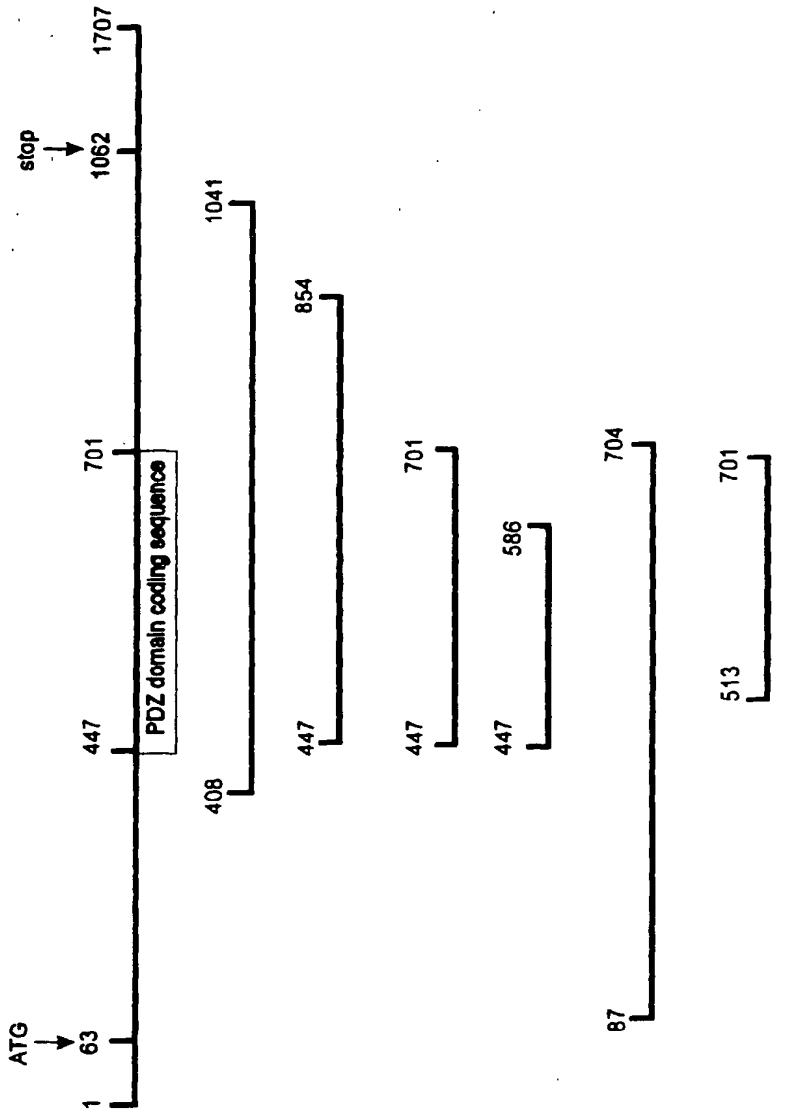


Fig. 4



European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 98 12 2992

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X,D	WO 97 27296 A (HSC RES DEV LIM PTN (CA) UNIV TORONTO; ST.GEORGE-HYSLOP FRASER ROMMENS) 31 July 1997 (1997-07-31) * abstract * * page 23, line 16-20 * * page 47, line 29 - page 53, line 11 * * page 55, line 1-16 * * page 83, line 13-16 * Seq.ID:10 * page 111 * * page 113 - page 128; claims *	1-7	C12N15/12 C07K14/47 C12Q1/68 G01N33/68
X,D	ROUSSET R. ET AL.: "The C-terminus of the HTLV-1 Tax oncoprotein mediates interaction with the PDZ domain of cellular proteins" ONCOGENE, vol. 16, 1998, pages 643-654, XP002102450 * page 646; figure 2 * * page 651, right-hand column, paragraph 1 *	1-4	
X	DE VRIES L. ET AL.: "GIPC, a PDZ domain containing protein, interacts specifically with the C terminus of RGS-GAIP" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 95, October 1998 (1998-10), pages 12340-12345, XP002102451 * page 12342, left-hand column; figure 1 *	1-4	TECHNICAL FIELDS SEARCHED (Int.Cl.6) C12N C07K C12Q G01N

-/--

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
THE HAGUE	18 May 1999	Macchia, G

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
Y : particularly relevant if combined with another document of the same category
A : technological background
O : non-written disclosure
P : intermediate document

T : theory or principle underlying the invention
E : earlier patent document, but published on, or after the filing date
D : document cited in the application
L : document cited for other reasons

& : member of the same patent family, corresponding document

EPO FORM 1503 03.82 (P04C01)

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 98 12 2992

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
X	Database EMBL ID AF032120 Accession number AF032120 31 October 1998 81% identity with Seq.ID:1 nt.43-1450 95% identity between the deduced aminoacid sequence and Seq.ID:2 XP002102461 * the whole document * ---	1-4	
A	BUNN R.C. ET AL.: "Identification and characterization of a novel glucose transporter binding protein" FASEB JOURNAL, vol. 11, no. 9, 31 July 1997 (1997-07-31), page a969 XP002102452 Abstract 657 * abstract * ---	1-4	
X	Database EMBL ID AF061263 Accession number AF061263 11 November 1998 83% identity with Seq.ID:1 nt.41-1385 95% identity between the deduced aminoacid sequence and Seq.ID:2 XP002102462 * the whole document * ---	1-4	TECHNICAL FIELDS SEARCHED (Int.Cl.6)
A	LAMOTHE B. ET AL.: "Reexamining interaction of the SH2 domains of SYP and GAP with insulin and IGF-1 receptors in the two-hybrid system" GENE, vol. 182, no. 1-2, 5 December 1996 (1996-12-05), pages 77-80, XP004071933 * abstract * ---	1	
	-/--		

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
THE HAGUE	18 May 1999	Macchia, G

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EUROPEAN SEARCH REPORT

Application Number
Er 98 12 2992

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A	DEY B.R. ET AL.: "Cloning of human p55gamma, a regulatory subunit of phosphatidylinositol 3-kinase, by a yeast two-hybrid library screen with the insulin-like growth factor-I receptor" GENE: AN INTERNATIONAL JOURNAL ON GENES AND GENOMES, vol. 209, no. 1-2, 16 March 1998 (1998-03-16), pages 175-183, XP004114987 * abstract * ---	1	
A	BASERGA R.: "Controlling IGF-receptor function: a possible strategy for tumor therapy" TRENDS IN BIOTECHNOLOGY, vol. 14, no. 5, 1 May 1996 (1996-05-01), pages 150-152, XP004035784 * the whole document * ---	8-10	
A	BASERGA R.: "The Insulin-like Growth Factor I Receptor: a key to tumor growth?" CANCER RESEARCH, vol. 55, 15 January 1995 (1995-01-15), pages 249-252, XP002102458 * page 249, left-hand column, paragraphs 1,2 * * page 250, right-hand column, paragraphs 2,3 * * page 251, right-hand column * -----	8-10	TECHNICAL FIELDS SEARCHED (Int.Cl.6)

~~The present search report has been drawn up for all claims~~

Place of search	Date of completion of the search	Examiner
THE HAGUE	18 May 1999	Macchia, G

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& : member of the same patent family, corresponding document

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European Patent Office

Application Number
EP 98 12 2992

CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:

☒ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:

Claims 1-4 completely, 5-10 partially.